REMARKS/ARGUMENTS

Claims 1 to 15, 17 to 29, 31 and 53 to 68 are pending in the case. Claims 1 to 3, 5, 7, 8, 12, 13, 15, 17, 18, 19, 20, 22, 23, 25, 26, 27, 29 and 31 have been amended to more clearly present the invention and differences between the amended claims and the previously pending claims should not be viewed as acquiescence to any of the Examiner's rejections. New claims 53 to 68 have been added.

Amended claims 1, 8, 15 and 18 and new claim 65 contain the feature of microinjecting a "DNA molecule", support for which is provided, for example, at the last sentence of paragraph [0135] where it is stated that "A recombinant DNA molecule of the present invention can be transferred into the extra-chromosomal or genomic DNA of the host." Amended claim 20 and new claims 55 and 65 have the feature of isolating the protein from an egg of a chick that tests positive for the transgene developed to sexual maturity, support for which is found, for example, in originally filed claim 20 and, for example, in the second sentence of paragraph [0147] at page 64 of the specification where it is stated that "In a preferred embodiment, the protein is produced and isolated from an avian egg." New claims 55 and 65 have the feature of a "pharmaceutical protein" which is supported for example, in the first sentence of paragraph [0145] page 63 of the specification where it is stated that "The present invention can be used to express, in large yields and at low cost, a wide range of desired proteins including those used as human and animal pharmaceuticals, diagnostics, and livestock feed additives." New claim 54, 64 and 66 specify specific pharmaceutical proteins which is supported, for example, at the second sentence of paragraph [0145] at page 63 where it is stated that "Proteins such as growth hormones, cytokines, structural proteins and enzymes, including human growth hormone, interferon, lysozyme, and β-casein, are examples of proteins that are desirably expressed in the oviduct and deposited in eggs according to the invention. Other possible proteins to be produced include, but are not limited to, albumin, α-1 antitrypsin, antithrombin III, collagen, factors VIII, IX, X (and the like), fibrinogen, hyaluronic acid, insulin, lactoferrin, protein C, erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), tissue-type plasminogen activator (tPA), feed additive enzymes, somatotropin, and chymotrypsin. Immunoglobulins and genetically engineered antibodies, including immunotoxins that bind to surface antigens on human tumor cells and destroy them, can also be expressed for use as pharmaceuticals or diagnostics. It is contemplated that immunoglobulin polypeptides expressed in avian cells following transfection by the methods of the present invention may include monomeric heavy and light chains, single-chain antibodies or multimeric immunoglobulins comprising variable heavy and light chain regions, *i.e.*, antigen-binding domains, or intact heavy and light immunoglobulin chains." Amended claims 7 and 23 and new claims 60 and 67 refer to use of the ovalbumin, lysozyme, ovomucoid, ovomucin, conalbumin and ovotransferrin transcriptional regulatory elements, which is supported, for example, at paragraph [0100], at page 51 of the specification where it is stated that "The promoter may optionally be a segment of the *ovalbumin* promoter region that is sufficiently large to direct expression of the coding sequence in the tubular gland cells. Other exemplary promoters include the promoter regions of the *ovalbumin*, *lysozyme*, *ovomucoid*, *ovotransferrin* or *ovomucin* genes…". New claim 53 which specifies that the nucleic acid is DNA is supported for example, at paragraph [0036] of page 11, which states that "The term "nucleic acid" as used herein refers to any natural and synthetic linear and sequential arrays of nucleotides and nucleosides, for example cDNA, genomic DNA, mRNA, tRNA, oligonucleotides, oligonucleosides and derivatives thereof." Amendment to claim 2 and new claim 56 are supported, for example, in the second to last sentence of paragraph [0035] at page 11 where it is stated that "For example, cells obtained from a stage later than a stage I embryo, up to and including a stage X embryo, *i.e.*, stages II-X, may be useful in the present invention." New claims 57, 58, 59, 61, 62 and 63 are supported, for example, in originally filed claims 3, 5, 6, 8, 9 and 10 respectively.

Applicant has made amendments to certain claims to make the claims more clear. For example, claims 13 and 27 have been amended to delete the term "selected"; claim 3 has been amended changing "the white" to "egg white"; claim 12 has been amended to depend from claim 1; and claim 29 has been amended to specify "nucleic acid" instead of "nucleotide sequence", each to make the claims more clear. To provide consistency, the term "said" in the claims has been changed to "the" which has the same meaning as said. The second usage of the term "comprising" in claims 1 and 20 has been changed to "containing", which has the same meaning as comprising, to avoid repetitive use of the term comprising. Applicant has amended the specification to update the status of cited applications in accordance with the Examiner's comments. A typographical error in an application number and filing year has been corrected in paragraph [0251] at page 92 of the specification. This same (correct) application number and filing date is referred to in paragraph [0149] at page 64 of the specification. This amendment includes no new matter.

The Examiner objects to the specification under 37 CFR 1.821 through 1.825 stating that the sequences in the specification and the Drawings have inconsistencies. In particular, the Examiner states that SEQ ID NO: 6 in Fig. 1 is not the same as SEQ ID NO: 6 in Fig. 15 and that SEQ ID NO: 5 in Fig. 2 is not the same a SEQ ID NO: 5 in Fig. 15 and that none of the SEQ ID NOs in 1-6 and 14 correlate to the SEQ ID NOs in Fig. 15. Applicant provides herewith replacement sheets for Fig. 15 having amended SEQ ID NOs. Applicant believes the amended Fig. 15 remedies the inconsistencies pointed out by the Examiner. The Examiner also states that SEQ ID NO: 42 (claim 16 and 30) does not exist. Applicant believes SEQ ID NO: 42 does exist and is included in the sequence listing filed along with the application on 10/2/03; however, claims 16 and 30 have been canceled obviating the objection.

In accordance with the Examiner's request, copies of Declarations of Dr. Christmann and Dr. Leavitt filed in the parent application 10/251,364 are included with this response. A copy of the Declaration of Dr. Dodgson filed in the parent application is also included for the Examiner's consideration.

The Examiner rejects the claims under 35 USC 112, first paragraph. This is an enablement rejection. The Examiner states that the specification, while being enabling for producing transgenic avians comprising microinjecting into a cell of an avian embryo a nucleic acid comprising a transgene comprising a nucleotide sequence encoding a heterologous polypeptide; introducing the microinjected avian embryo into an oviduct of a recipient hen, such that the recipient hen lays a shelled egg containing the microinjected avian embryo; incubating the shelled egg containing the microinjected avian embryo until the egg hatches; testing a hatched chick for the presence of the transgene; and developing a chick that tests positive for the transgene to sexual maturity, that the specification does not provide enablement for making a transgenic avian by merely incubating a shelled egg containing the microinjected avian embryo until hatch, using any "tissue-specific promoter" or "magnum-specific promoters," a "heterologous polypeptide… …optimized for codon usage by an avian," a transgene comprising "at least two nucleotide sequences each encoding a heterologous polypeptide," "that form a multimeric protein," that "specifically bind a selected ligand," that is an "antibody," for using BAC to obtain transgenic avians.

Applicant traverses the rejection; however, amendments to the claims have obviated aspects of the Examiner's rejections. For example, the claims as amended conform to the Examiner's comments regarding enablement since, for one thing, the claims essentially contain the elements of producing transgenic avians comprising microinjecting into a cell of an avian embryo a nucleic acid containing a transgene comprising a nucleotide sequence encoding a heterologous polypeptide; introducing the microinjected avian embryo into an oviduct of a recipient hen, such that the recipient hen lays a shelled egg containing the microinjected avian embryo; incubating the shelled egg containing the microinjected avian embryo until the egg hatches; testing a hatched chick for the presence of the transgene; and developing a chick that tests positive for the transgene to sexual maturity, which, as the Examiner states, is enabled by the application.

The Examiner states that the specification does not enable any "tissue specific promoter" or "specific for the magnum" as is claimed. Applicant disagrees with the Examiner since use of an ovomucoid promoter has been demonstrated in the specification. See, for example, Example 37 and Example 38 spanning from pages 107 to 110 which disclose the use of a tissue specific (i.e., magnum specific) ovomucoid promoter, in accordance with the invention.

The Examiner states that the specification does not enable a transgene comprising "at least two nucleotide sequences each encoding a heterologous polypeptide," "that form a multimeric protein," that "specifically bind a selected ligand," that is an "antibody". Applicant disagrees since Examples 37 and 38 provide for the production of such a heterologous polypeptide (i.e., an antibody).

The Examiner states that the specification does not provide enablement for a "heterologous polypeptide... ...optimized for codon usage by an avian. Applicant disagrees since, for one thing, codon optimization is well understood in the field of molecular biology. For example, see the discussion below regarding a coding sequence that is optimized for codon usage by an avian.

The Examiner states that the specification does not enable using BAC expression vectors to make trasnsgenic avians and that the art at the time of filing was limited to making transgenic chickens using plasmids or viral vectors. Applicant disagrees because, for one thing, the specification provides functional examples of the production of a transgenic avian using a BAC. See, Example 37 and Example 38 spanning from pages 107 to 110 of the specification.

The Examiner rejects the claims under 35 USC 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The specific rejections are as follows

The Examiner states that Claim 1 is indefinite because the method steps merely require hatching a microinjected avian embryo but does not result in a chick comprising the transgene and that the claim should clearly set forth obtaining a chick that comprises the transgene. Claim 1 has been amended thereby obviating this rejection.

The Examiner states that claim 2 is indefinite because the metes and bounds of an "early stage" embryo cannot be determined and that one of skill would not be able to determine which stages applicants consider "early" stages of development. Claim 2 has been amended thereby obviating this rejection.

The Examiner states that claim 3 is indefinite because the metes and bounds of a developing egg cannot be determined and that it is unclear when an egg is no longer "developing" and that deleting "developing" would overcome this rejection. "Developing" has been deleted in accordance with the Examiner's comments.

The Examiner states that claim 6 is indefinite because the metes and bounds of a "tissue specific promoter" are not defined in the specification or the art at the time of filing. Applicant disagrees with the rejection since the meaning of "tissue specific promoter" is well known in the field of molecular biology. For example, a Google search of "tissue specific promoter" performed on January 18, 2008 yielded over 180,000 hits.

The Examiner states that claim 7 is indefinite because the metes and bounds of a "tissue specific promoter" "specific for the magnum" are not defined in the specification or the art at the time of filing. Applicant disagrees with the rejection; however, claims referring to a magnum specific promoter have been canceled thereby obviating the rejection.

The Examiner states that claim 8 is unclear because it cannot be determined how the nucleotide sequence is associated with an NLS during microinjection and that the nucleotide sequence may comprise a nucleic acid sequence encoding an NLS but it is not combined with an NLS peptide prior to microinjection as claimed. Claim 8 has been ameded to make clear that the nucleic acid, i.e., DNA molecule, is *mixed* with the NLS, which is supported, for example, at the first sentence of paragraph [0118] at page 56 where it is stated that "Targeting of the nucleic acids introduced into embryonic cells using methods of the invention may be enhanced by mixing the nucleic acid to be introduced with a nuclear localization signal (NLS) peptide prior to introduction, *e.g.*, microinjection, of the nucleic acid."

The Examiner states that claim 9 is indefinite because the metes and bounds of a coding sequence that is "optimized for codon usage by an avian" are unclear and that the metes and bounds of such coding sequences cannot be envisioned, i.e., the structure of such coding sequences and the process used to optimize such coding sequences cannot be envisioned. Applicant disagrees with the rejection since codon optimization for a particular tissue or organism was well known in the field of molecular biology prior to the filing date of the application. For example, the prior art is rife with evidence that the field was aware of the concepts of preferential codon usage and optimization of codons for increased expression of a heterologous sequence in a particular organism. Countless reports of preferential codon usage exist for specific organisms. One of the earliest comes from Elton *et al.* (1976), *J. Mol. Evol.* 8:117-135, where the authors describe the "hypothesis that rapidity of translation of mRNAs in the E. coli system is dependent on ... codon usage patterns" (**Exhibit 1**). Given this reference, a person of skill in ***1976*** would recognize that if she were to express a heterologous protein in *E. coli*, she should optimize the codons of the heterologous coding sequence accordingly.

Applicants draw the Examiner's attention to three broader reports that also teach the concept of codon usage optimization. First, Ikemura (1985), *Mol. Biol. Evol.* 2:13-34, reviews codon choice patterns in both unicellular and multicellular organisms. He correlates codon usage and protein production levels (**Exhibit 2**). Sharp and Li (1986), *Nuc. Acids Res.* 15:1281-1295, make a similar correlation, stating that "there is a clear positive correlation between degree of codon bias and level of gene expression" (**Exhibit 3**, page 1281). The authors continue, emphasizing the benefit of chemically synthesizing a "new gene to include more appropriate codons" for increased expression of mammalian (*i.e.*, heterologous) genes in *E. coli* or yeast (**Exhibit 3**, page 1289). Finally, Nakamura *et al.* (2000), *Nuc. Acids Res.* 28:292, describe a publicly accessible website that relies in part on the exemplary program provided by the specification at page 24 (**Exhibit 4**). As of 2000, the website (www.kazusa.or.jp/codon/) provided a compilation of the preferred codons for 8792 organisms. **Exhibit 5** represents the relevant pages from the website that were updated in August 2000, demonstrating that the codon usage tables for several avian species were available as of the filing date. Applicants have included a few examples: *Gallus sp.* (chicken), *Anus sp.* (duck), *Anser anser* (a goose species), and *Coturnix coturnix* (a quail species). The report and website provide further evidence that the field of molecular biology was conversant with the idea of optimizing the codons within a heterologous coding sequence for improved heterologous protein expression.

The methods of the invention utilize avian cells for expression of heterologous sequences, analogous to the *E. coli* expression systems described in Elton, Ikemura, and Sharp. Given the teachings of the prior art, one of skill in molecular biology as of the filing date, ***twenty-five*** years later, would clearly understand that the codons used in the heterologous coding sequence would affect expression levels. The skilled artisan would be able to envision a codon optimized for expression in an avian, and in fact, could find scores of optimized avian codons on the publicly available website described in **Exhibit 4**. Alternatively, one could use the exemplary optimizing program and coding sequences provided in the specification to design a nucleotide sequence comprising a codon optimized for expression in an avian.

In summary, "optimized for codon usage by an avian" clearly defines the metes and bounds of the claimed subject matter. On the other hand, the Examiner has failed to provide any explanation of why one of skill would not be able to envision the claimed method. The concept of optimizing codon usage for expression in an avian, or any other organism, would be clear to a person of ordinary skill in molecular biology and the teachings of the prior art provide evidence that the claim language and concept would be clear to one of skill in the art. Accordingly, claim 9 complies with the definiteness requirements laid out in the MPEP.

The Examiner states that claim 16 is indefinite because use of the parentheses makes the claim unclear. In particular, it is unclear if SEQ ID NO: 42 is included in the claim and the sequence listing does not include SEQ ID NO: 42 and that Claims 16 and 17 are indefinite because "a heterologous polypeptide-encoding sequence" in claim 16 does not further limit the heterologous polypeptide in claim 1. Claim 16 has been canceled obviating the rejection.

The Examiner rejects claims 1-9, 11 and 18 under 35 USC 103(a) as being unpatentable over Tanaka et al, J. Reprod. Fert. (1994) 100: 447 to 449 (Tanaka) in view of Naito, Transgenic Animals: Generation and Use, Harwood Academic Publishers, p. 69 to 73 (Naito) and Love et al, Bio/Tech. (1994) 12: 60 to 63 (Love). Applicant traverses this rejection.

The Examiner states that Tanaka taught delivering DNA to a zygote before delivering the zygote to the oviduct of a recipient hen and that the zygote hatched and became a chick. Applicant disagrees with the Examiner's characterization of Tanaka.

Tanaka teaches the *in vitro* fertilization of an avian ovum and transfer of the fertilized ovum (zygote) to the oviduct of a hen and the subsequent laying of a hard shell *in vitro* fertilized egg (See Fig. 1 of Tanaka). The Examiner refers to the last full paragraph of Tanaka in which the author refers to a preliminary attempt to incorporate a linearized *MitoZ* gene into avian embryos. The author indicates that in the 20 shelled eggs obtained in the experiment three eggs hatched and three embryos died during the incubation and that one embryo expressed *MitoZ* but the hatchlings did not. Tanaka goes into little detail in explaining how the DNA was introduced into the embryo or other specifics regarding the experiment. However, one thing that is clear to applicant is that the preliminary work of Tanaka was a failure. That is, no transgenic avians were identified by Tanaka using a procedure in which an embryo is implanted into an avian oviduct. In addition, no information was obtained which would indicate that a DNA introduced into an avian in such a manner could become incorporated into the germline. Therefore, Tanaka discourages or teaches away from producing transgenic avians using methods of embryo reimplantation providing no motivation to combine the teachings of Naito or Love with that of Tanaka.

In view of the teaching away of Tanaka and lack of motivation to combine, the present claims are not made obvious by Tanaka and the cited reference.

The Examiner rejects claims 1-14, 18-22, 25-27 and 31 under 35 USC 103(a) as being unpatentable over Ivarie et al, US Patent No. 6,730,822 (Ivarie) in view of Tanaka. Applicant traverses the rejection.

In Ivarie, as in Harvey below, embryo cells are transduced by introducing viral particles into the egg that has progressed to the stage of being encapsulated in a hard shell which is laid by the hen (see, for example, Example 3 of Ivarie which cites to Thorval et al Transgenic Research (1995) 4; 369 to 376). After transduction, such hard shell eggs require only standard incubation, for example, in a commercial incubator until hatch. It would be counter-intuitive an of no apparent use to reintroduce an egg which has been laid by the chicken back into a chicken for *in ovo* incubation as required in the pending claims. In addition, in Ivarie, virus particles are introduced into the subgerminal cavity which thereafter infect the embryo cells, as in Harvey below.

In view of the distinguishing features of the present invention over Ivarie and the teaching away of Tanaka, applicant submits that the present claims are not made obvious by Ivarie in combination with the cited reference.

The Examiner rejects claims 1-7, 9, 11-13, 19-22 and 25-27 over Harvey et al, Nature Biotech (2002) 19: 396 to 399 (Harvey) in view of Tanaka. Applicant traverses the rejection.

Harvey discloses transducing embryos (fertilized ova) in freshly laid eggs by introducing viral particles through a hole in the shell of the egg and sealing the egg (See the Experimental Protocol of Harvey which cites Harvey et al (2002) Poultry Science 81: 202 to 212, copy included with this response). Harvey discloses use of an embryo that has progressed to the stage of being encapsulated in a hard shell egg which is laid by the hen before being transduced. After transduction, such hard shell eggs require only standard incubation in a commercial incubator until hatch. It would be counter-intuitive and of no apparent use to reintroduce an egg which has been laid by the chicken back into a chicken for *in ovo* incubation as required in the pending claims. The specification of the present invention discloses the egg being injected with nucleic acid after being surgically removed from the avian (see, for example, the specification page 95, paragraph [0270]).

In addition, the present invention discloses the introduction of nonviral DNA into an embryo, such as plasmids and BACs to produce transgenic avians by injection. There is no reason to expect that vectors such as these could be introduced into the avian embryo by employing the methods of Harvey. As the Examiner notes, in Harvey the virus is introduced into the subgerminal cavity which is a space below the cells of the embryo. When the virus is placed in this cavity in proximity to the embryo it is able to infect the embryo cells. That is, the virus passes into the embryo cells by standard mechanism of infection which is not available to noninfective nucleic acids such as BACs or plasmids.

In view of the distinguishing features of the present invention over Harvey and the teaching away of Tanaka, the present claims are not made obvious by Harvey in combination with the cited reference.

The Examiner states that applicant's claim for domestic priority under 35 USC 119(e) is acknowledged, but that the 60/351550 provisional application upon which priority is claimed fails to provide adequate support under 35 USC 112 for the pending claims. The Examiner cites a passage from the '550 provisional which states that:

> Approximately two nanoliters of DNA were injected into the germinal disk of stage I (E,G.&K,) White Leghorn embryos obtained two hours after oviposition of the

> previous egg. DNA amounts per injection ranged from 10 picograms to 400 picograms. Injected embryos were surgically transferred to recipient hens, and hard shell eggs were incubated and hatched...

and states that the '550 provisional application did not teach transferring the embryo into the oviduct: therefore, the effective filing date of the claims is 9-18-02. Applicant traverses the Examiner's assignment of priority in the case because, for one thing, a skilled artisan reading the procedure would instantly understand that the injected embryo was transferred to the oviduct of the avian. In addition, the 11th and 12th to last pages of the provisional application includes the following, step c) of which specifies that the microinjected embryo is introduced into the oviduct of a recipient hen.

1. A method of producing a transgenic avian comprising the steps of:
   a) isolating a stage I avian embryo having a germinal disc from an oviduct of a fertilized hen;
   b) microinjecting a selected nucleic acid into the avian embryo, comprising the steps of:
      (i) introducing said needle containing the selected nucleic acid into the germinal disc cytoplasm of said avian embryo;
      (ii) aspirating into an injection needle a selected nucleic acid comprising a promoter and a gene of interest;
      (iii) introducing said needle containing the selected nucleic acid into the germinal disc cytoplasm of said avian embryo;
      (iv) injecting said nucleic acid into the germinal disc cytoplasm; and
      (v) withdrawing said needle from the avian embryo;
   c) introducing said microinjected avian embryo into an oviduct of a recipient hen,
   d) allowing said recipient hen to develop and lay a shelled egg containing the microinjected avian embryo having the selected nucleic acid therein; and
   e) incubating the egg containing the microinjected avian embryo for sufficient time to hatch a chick having the selected nucleic acid therein.

Therefore applicant is entitled to the benefit of priority of the '550 application.

The Examiner rejects claims 1-15 and 18-29 under the judicially created doctrine of obviousness type double patenting as being unpatentable over certain copending patent applications.

The Examiner rejects claims 1-15 and 18-29 under the judicially created doctrine of obviousness type double patenting as being unpatentable over claims 1-41 of copending application No. 10/251,364 in view of the disclosure of '364. Applicant includes herewith an appropriate Terminal Disclaimer over the now issued 10/251,364 application, US Patent No. 7,312,374.

The Examiner rejects claims 1-15 and 18-29 under the judicially created doctrine of obviousness type double patenting as being unpatentable over claims 1-41 of copending application No. 10/842,606, in view of the disclosure of '606. Applicant traverses the rejection, because for one thing, the '606 application does not have claims directed to subject matter claimed in the present case. Therefore the rejection should be withdrawn.

The Examiner rejects claims 1-15 and 18-29 under the judicially created doctrine of obviousness type double patenting as being unpatentable over claims 1-41 of copending application No. 11/337,361, in view of the disclosure of '361. Applicant traverses the rejection, because for one thing, the '361 application does not have claims directed to subject matter claimed in the present case. Therefore the rejection should be withdrawn.

The Examiner rejects claims 1-15 and 18-29 under the judicially created doctrine of obviousness type double patenting as being unpatentable over claims 1-41 of copending application No. 11/376,023, in view of the disclosure of '361. Applicant traverses the rejection, because for one thing, the '023 application does not have claims directed to subject matter claimed in the present case. Therefore the rejection should be withdrawn.

The Examiner rejects claims 1-15 and 18-29 under the judicially created doctrine of obviousness type double patenting as being unpatentable over claims 1-41 of US Patent No. 6,730,822, in view of the disclosure of '822 and Tanaka. Applicant traverses the rejection. This double patenting rejection is improper and should be withdrawn because, for example, for the reasons provided for nonobviousness under 35 USC 103(a) for Ivarie in view of Tanaka.

In conclusion, applicant believes that pending claims 1 to 15, 17 to 29, 31 and 53 to 68 meet the requirements for patentability under 35 USC 112, first and second paragraphs and that the claims are nonobvious under 35 USC 103(a). Therefore, applicant submits that the presently pending claims are allowable and respectfully requests the Examiner to pass the above-identified application to allowance.

Any fees required for submission of this Response are authorized by applicant. Please deduct any fees and credit any overpayment to Deposit Account No. 501729.

If any issues remain to be addressed in this matter, which might be resolved by discussion, the Examiner is respectfully requested to call applicants' undersigned counsel at the number indicated below.

Respectfully submitted,

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